10.1053.paor.2000.0206 available online at http://www.idealibrary.com on IDEAL

ARTICLE

Unique Morphological Alterations of the HTLV-I Transformed C8166 Cells by Infection with HIV-1

Joseph ONGRÁDI,[1,3,4] Helen M LAIRD,[2] Joseph F SZILÁGYI,[3] Attila HORVÁTH,[1] Mauro BENDINELLI[4]

[1]National Institute of Dermato-Venerology, Budapest, Hungary, [2]Department of Veterinary Pathology and [3]Institute of Virology, University of Glasgow, Glasgow, UK, [4]Department of Biomedicine, University of Pisa, Pisa, Italy

C8166 cells express T lymphocyte markers, a monocyte-specific esterase, *tax* polypeptide of HTLV-I. In spite of this transactivator, their HIV-1 yield is low. Their culture conditions were modified, and infected cells were immobilized on a poly-L-lysine sheet under semisolid overlays to study their phenotypic alterations and HIV-1 production by microscopy and electron microscopy. Another lymphoid cultures (MT-4, CEM, CEM-ss, AdCEM) similarly treated were infected with either HIV-1/RF or IIIB. Specificity of HIV-1 was compared to the effects of vesicular stomatitis virus (VSV). Unlike other cultures, HIV-1/RF infected C8166 cells in Eagle's MEM exhibited surface projections resembling hairy leukemia cells, which was followed by balloon degeneration and apoptosis. Immobilized HIV-1 infected cultures formed flat syncytia with several interdigitating dendritic projections. Syncytia shrunk with condensed nuclear material and axon-like filaments characteristic for infected macrophages. VSV induced enlargement and necrotic lysis of all cell types. Early postinfection with HIV-1, electron microscopy revealed irreversible membrane fusion above cell nuclei, and transient fusion between filaments. Transient presence of coated vesicles containing intact HIV-1 particles, Birbeck granule-like structures of Langerhans cells, fibrillar-lamellar structures resembling hairy leukemia or Sézary cells were detected. Late postinfection, high proportion of HIV-1 bud from polarized cytoplasm was empty particle, while that bud and entrapped in cytoplasmic vacuoles contained two or multiple cores in a fused envelope. The effect of early gene products of HIV-1 on HTLV-I and C8166 cells might elicit their latent potentials for monocyte or interdigitating dendritic cells, while in the later phase HTLV-I products might alter HIV-1 virion assembly. (Pathology Oncology Research Vol 6, No 1, 27–37, 2000)

Keywords: C8166, HIV-1, leukemia, surface projections, cellular mimicry, aberrant virions

Introduction

C8166 cells are among the most commonly used permanent lymphoid lines to quantitate lymphotropic strains of HIV-1[15, 36] based upon their high sensitivity and extensive syncytium formation within a short time postinfection.[5,6,8,43] These cells form clusters in suspension cultures and express surface markers of activated T cells on their surface,[43] but do not possess monocyte/macrophage specific surface molecules.[5] However, it has been known but ignored for a long time that they contain a non-specific esterase, which is characteristic for macrophages.[18] The C8166 cell line is a subclone of CR63/R_{II}-4 cells[39] derived by *in vitro* fusion of primary umbilical cord blood cells and HTLV-I producing cells obtained from a patient with adult T cell leukemia-lymphoma. They contain at least one HTLV-I provirus that express the p40 *tax* gene, only, but lack expression of *gag* proteins, reverse transcriptase activity and virus particles in the culture medium.[42] In spite of their high HIV-1 sensitivity, the yield of infectious HIV-1 in their supernatant is found to be low as compared to other lymphoid lines (e.g. H9).[27] This is surprising, because the *tax* polypeptide of HTLV-I is regarded as one

Received: June 10, 1999; *revised:* Nov 6, 1999; *accepted:* Jan, 10, 2000
Correspondence: Dr. Joseph ONGRÁDI, National Institute of Dermato-Venereology, Mária utca 41., 1085 Budapest, Hungary; Tel: +361-266-0465/ext.5717; fax: +361-267-6974
E-mail: ongjos@hotmail.com

Abbreviations: EMEM – Eagle's minimal essential medium; FCS – fetal calf serum; HIV-1 – human immunodeficiency virus type 1; HTLV-I, HTLV-II – human T-cell leukemia virus type I, type II; LTR – long terminal repeat; MIP – macrophage inhibitory protein; m.o.i. = multiplicity of infection; PFU/ml – plaque forming units/ml; p.i. – postinfection; PLL – poly-L-lysine; RANTES – regulated on activation normal T cell expressed and secreted; VSV – vesicular stomatitis virus

 1219-4956/00/010027+11 $ 12.00/0

of the most potent transactivators of HIV-1 LTR acting through nuclear transcriptional factors of the cell.[1,8,13,19] It was estimated that HIV-1 uses nearly all of the cell's capacity to make RNA and an excessive amount of viral proteins.[6,19] Replication kinetics in a single growth cycle showed that the majority of newly formed virus was cell-associated[8]. Electron microscopy detected HIV-1 budding into both the medium and intracytoplasmic vacuoles,[15] which latter phenomenon with entrapped virions is known to occur in macrophages.[12,23] The fact that C8166 cannot be regarded as a conventional T cell line, recently has been challenged. It has been reported lately that, unlike H9 cells, they express all three receptors for the Fc portion of immunoglobulins, namely FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16),[27] which are known potentially to bind and transmit non-neutralized HIV-1 into CD4 negative cells.[23] Molecules belonging to the G-protein coupled chemokine receptor family have been identified as coreceptors for HIV. Preference of HIV strains to bind different coreceptor molecules determines their macrophage or lymphocyte tropism.[9,17] C8166 cells possess CXCR-4 chemokine receptors (characteristic for lymphocytes), but after treatment with β-chemokines (MIP-1α, β-1, RANTES) these cells also become slightly positive for CCR-5 coreceptors (specific for macrophages).[9] Another alternative HIV-1 receptor, APJ was found on C8166 cells, but not on CEM-ss and other lymphoid and macrophage cultures. APJ is widely expressed in the human brain and NT2N neurons, a frequently used *in vitro* model for human neurons.[10] Neural and immune cells might be related in their differentiating pathways: under the influence of soluble environmental signals, lately, neural stem cells were found to produce a variety of blood cell types including myeloid and lymphoid cells.[2]

So far, no corresponding morphological and etiological explanations of these findings have been given. Environmental stimuli among them oncogenic viruses are known to modify the function and phenotypic properties of cells. To explore such potentials of C8166 cells, their culture conditions were modified. Adherence is characteristic for both monocytes/macrophages and neurons *in vivo* and *in vitro*, therefore C8166 cells were immobilized to the culture vessels, subsequently infected and covered with semisolid overlays. Their morphological alterations upon infection were compared to similarly treated other lymphoid cultures with and without HTLV carriership. To establish whether the cytopathic effects in immobilized cells are specific for HIV-1, comparison was made with the effect of VSV, another enveloped virus, that is extensively used in pseudotype formation with HIV-1 and HTLV-I,[5,14] but its effect on immune cells has not been studied yet. In this report, the structural alterations of cells and viruses produced are described following time course of infection.

Materials and Methods

Cells and viruses

HIV-1 lymphotropic strains RF and IIIB were produced in suspension cultures of H9 cells, designated as H9/RF and H9/IIIB, respectively. Both uninfected and infected cultures were maintained by weekly passages at $1x10^6$ input cells per ml in either RPMI-1640 (Whittaker Bioproducts, Waltersville, MD, US) or EMEM (Media Laboratory, Institute of Virology, Glasgow, UK) containing 10% fetal calf serum (Gibco, Paisley, UK), 2 mM L-glutamine (Gibco), 20 mM HEPES (Sigma, St.Louis, MO, US), 40 μg/ml gentamicin (Gibco) or 10 μg/ml ciprofloxacin (Bayer UK Ltd., Newbury, UK). Supernatant fluids of 24h old cultures obtained by low speed centrifugation and filtered through a 0.45 μm pore size filter (Millipore, Bedford, MA, US) served as cell free virus stocks and their aliquots were frozen below –70°C.

Suspension cultures of other lymphoid cells as CEM, CEM-ss, and two HTLV-I transformed lines, C8166 and MT-4 were maintained in the same way. An adherent subline of CEM, AdCEM and fibroblast BHK-21 C-13 cells were maintained in EMEM and split by trypsinization (0.05% trypsin and 0.02% Versene, Flow Laboratories, Irvine, UK). For infection, $2x10^4$ lymphoid cells, $4x10^4$ AdCEM or BHK-21 cells in 150 μl EMEM were seeded in flat bottomed 96 well tissue culture plates (Costar, Cambridge, MA, US), and next day their medium was adjusted to 200 μl by inocula of HIV-1 strains for 90 min with subsequent washes. Infected cells were designated as CEM/RF, CEM/IIIB, etc. The titre of HIV-1 was quantitated by syncytium scoring after transferring 50 μl supernatant fluid onto $5x10^4$ PLL (70,000 to 150,000 MW, 100 μg/ml) bound CEM-ss cells in 50 μl RPMI-1640 medium.[30,31,33]

VSV Indiana serotype was produced in 2 liter roller cultures (Falcon, Becton-Dickinson, Parsipany, NJ, US) of BHK-21 cells, subsequently concentrated by ultra-centrifugation, kept in aliquots below –70°C as described.[32] It titered at $2.5x10^9$ PFU/ml. BHK-21 and AdCEM monolayers as well as C8166 suspensions in microwells were infected at m.o.i. 0.1 in the same way as mentioned for HIV-1. Cytopathology was observed and photographs were taken using an Olympus microscope.

Monolayer cultures of lymphoid cells

$2x10^4$ C8166, CEM-ss, MT-4 or H9 cells in 150 μl media were bound to the flat bottom of 96 well tissue culture plates by PLL for 60 min, then their media was adjusted to 200 μl by virus stocks for another 60 min.[27] After aspirating and washing, the media were replaced by 100 μl of semisolid overlays: in the case of CEM-ss cultures 0.6% agarose (Standard low m_r, Biorad, Watford, UK) or 1% methyl-cellulose (Met-2000, Eurobio, Paris, France) dissolved in complete

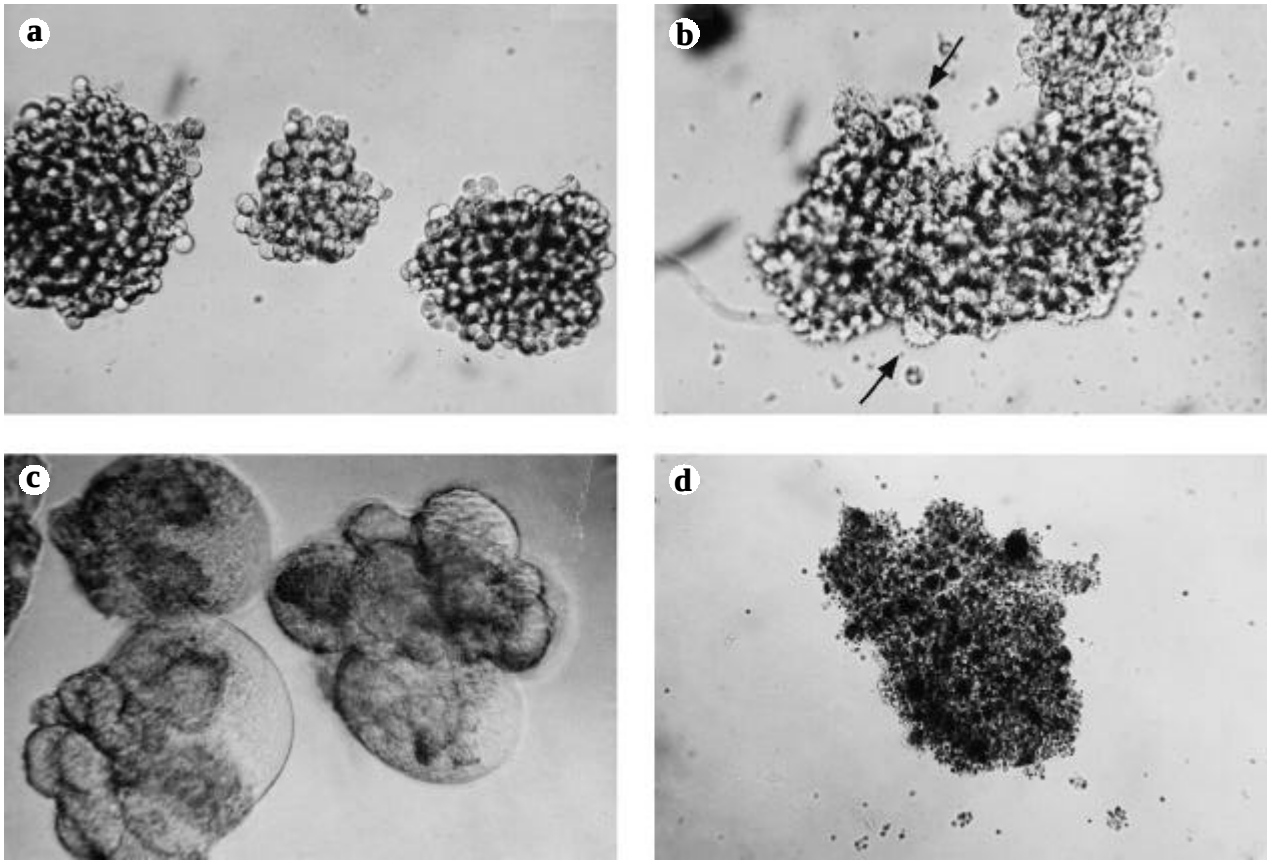

Figure 1. The course of HIV-1/RF infection in suspension cultures of C8166 cells. a. 24h old normal cell clusters before infection (50x). b. Hairy cells (arrows) at 12h p.i. (50x). c. Typical syncytia balloon degeneration and reaching its peak at 48h p.i. (100x). d. Collapsed syncytia with residual cell debris at 120h p.i. (50x).

RPMI-1640 with 20% FCS was used. In the case of C8166, MT-4 and H9 cultures, 0.6% agarose (SeaPlaque, LE grade, ICN Biomedical Ltd, High Wycombe, UK) dissolved in EMEM containing 20% FCS and 10 μg/ml ciprofloxacin but no phenol red was used. An appropiate second 100 μl overlay containing neutral red (Sigma) was applied on day 5 to stain cells before photography.

High resolution microscopy and electron microscopy studies

At various time intervals postinfection, $1x10^7$ C8166/RF cells in EMEM were gently mixed in 1:1 (v:v) with 8% glutaraldehyde in a 15 ml conical centrifuge tube, spun at 2000 rpm for 5 min and kept at 4°C overnight. Supernatant fluids were decanted and replaced by paraglutaraldehyde at 4°C for 120 min, then this was replaced by 0.1 M cacodylate buffer, pH 7.4 at 22°C for 15 min followed at 4°C overnight. Cell pellets were fixed with OsO_4, dehydrated and embedded in Araldite. For light microscopic studies, 1 μm sections were cut and stained with azure blue/methylene blue/borax solution.[29] For electron microscopy another ultrathin sections were stained with uranyl acetate and lead citrate.[30,33]

Results

Cytopathology of C8166 cells following HIV-1 infection

H9, C8166 and MT-4 cells were cultured and infected in EMEM as efficiently as in RPMI. Several individual uninfected C8166 and MT-4 cells in EMEM but not in RPMI adhered to the plastic, but without following cell division they died in a short time, leaving cell debris on the plastic, which was trypsin resistant.

The microscopic course of syncytium induction, balloon degeneration and cell death observed on resuspended C8166/RF (*Figure 1*), C8166/IIIB, MT-4/RF, MT-4/IIIB, CEM/RF, CEM/IIIB, CEM-ss/RF, CEM-ss/IIIB cultures were the same. C8166/RF differed in the cytopathology that developed from the other HIV-1 infected cells, only in that they exhibited microscopically well visible hair-like structures on their surface between 12 and 24 h p.i. (*Figure 1 b*). Observation of thin sections at higher magnification revealed that these "hairs" were protoplasmic extrusions. These structures existed on the surface opposite to nuclei of the developing syncytia (*Figure 2 a-c*), but disappeared as the cells began to balloon and take on a smooth surface

(*Figure 1 c, Figure 2 d-e*). Nuclei coalesced to form "U, V or C" shaped structures directly below one "pole" of the plasma membrane. At the opposite pole of large (>60 μm in diameter) syncytia, several vacuoles of various sizes were observed (*Figure 2 d*). Multiple, enlarged vacuoles formed huge balloons (*Figure 1*). Very early, the syncytia contained widened perinuclear cisternae and condensed chromatin (*Figure 2 b-d*) hallmarks of apoptosis. The latter manifest-

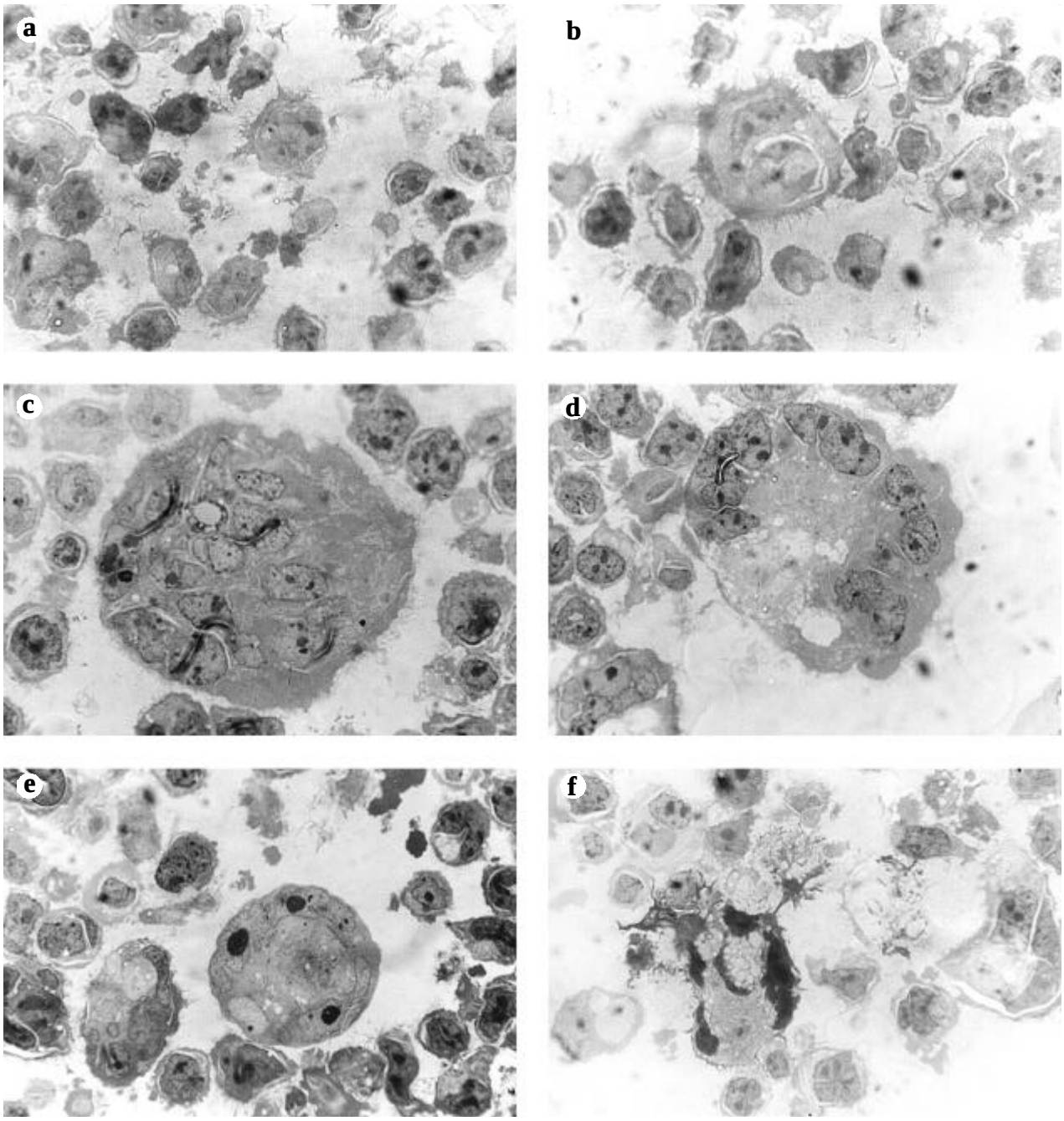

Figure 2. The effect of HIV-1/RF on the morphology of resuspended C8166 cells (1000x). a. Individual hairy cells dominate culture with enlarged nucleus at 12h p.i. b. Frequent fusion between cells at 24h p.i. Cytoplasmic vesicles appear. c. A large syncytium developed at 48h p.i. Both nuclei and cytoplasmic vesicles are located randomly. d. A large syncytium with relocated nuclei which are near the plasma membrane forming an U shape at 96h p.i. Condensation of nuclear material starts, while fine protolasmic projections still exist above nuclei. Vacuoles are relocated to the opposite pole of syncytium. e. A shrunken syncytium with 3 nuclei containing condensated chromatin at 120h p.i. Hairy projections disappeared, vacoules collapsed. f. A syncytium with completely disintegrating nuclei and protoplasm at 144h p.i. Round bodies inside cytoplasm might represent apoptotic bodies.

ed itself as dark inclusions located at the poles of the balloons (*Figure 1*). Finally, the balloons collapsed leaving only the inclusions as evidence of their former presence (*Figure 1*). Nuclei retained their location near the plasma membrane but now contained a single, large chromatin inclusion (*Figure 2 e*). Over the next several days the syncytia collapsed inwardly upon themselves with disintegration of both the protoplasm and nuclei (*Figure 2 f).*

Cytopathology in C8166 cells grown as a monolayer

During the initial 60 min binding period, C8166 cells formed a homogenous monolayer adhering to PLL (*Figure 3 a*). Infection with HIV-1/RF resulted in their unusual morphologic alterations. By 2 days p.i. cells showed evidence of fusion and formed interdigitating structures reminescent of dendritic cells. These projections of flat syncytia fused with adjacent cells (*Figure 3 b*). If the cells were grown with a few µl medium between the cells and the overlay the syncytia that formed were a mixture of the rounded type seen in cultures without an agarose overlay and the dendritic type (*Figure 3 c*). If no overlay was used the syncytia formed were all rounded with a "root" binding cells to the PLL-covered plastic. Although the flat syncytia collapsed over subsequent days, the plasma bridges remained well preserved. After 7–9 days the syncytia had shrunk so that the major evident structure was a mass of condensed nuclear material. Even at this time point some plasma and the plasma bridges between cells were visible as very fine dendritic or axonal filaments (*Figure 3 d*). There was no evidence of cell lysis until these cultures were discarded on day 14 p.i.

Cytopathology of HIV-1 and VSV in other lymphoid cultures

All lymphoid cell lines tested formed a homogenous monolayer adhered to PLL. Neither newly infected nor persistently infected H9 cells showed any sign of cyto-pathology when grown in monolayer under semisolid overlays. However, under both agarose and methylcellulose CEM-ss/IIIB cells formed small syncytia with smooth surface, which fused soon. Subsequently, large flat syncytia were formed, in which although the nuclei gathered near each other but were distributed randomly. Ballooning reached its maximal size under agarose on day 4 (*Figure 4 a*), under

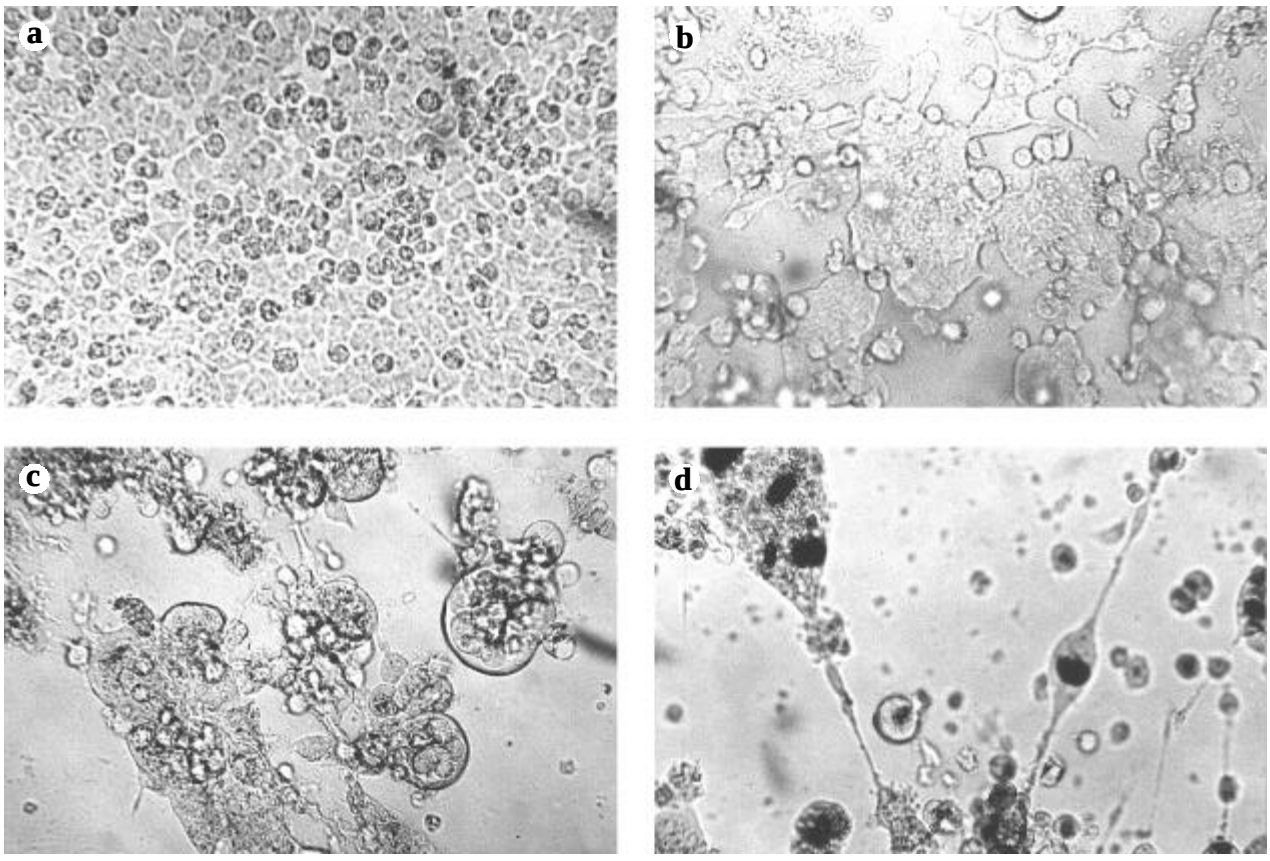

Figure 3. The course of HIV-1/RF infection in monolayer cultures of C8166 cells under agarose (50x). a. 48h old normal monolayer of cells bound to plastic wells by poly-L-lysine. b. Irregular large flat syncytia with interdigitating projections at 48h p.i. c. Several smaller syncytia fuse and retain the original shape of cells at 72h p.i. Plasma bridges become very thin. d. Collapsed syncytia with shrunken chromatin as a large dark inclusion at 192h p.i. Cytoplasmic bridges and projections become axon-like.

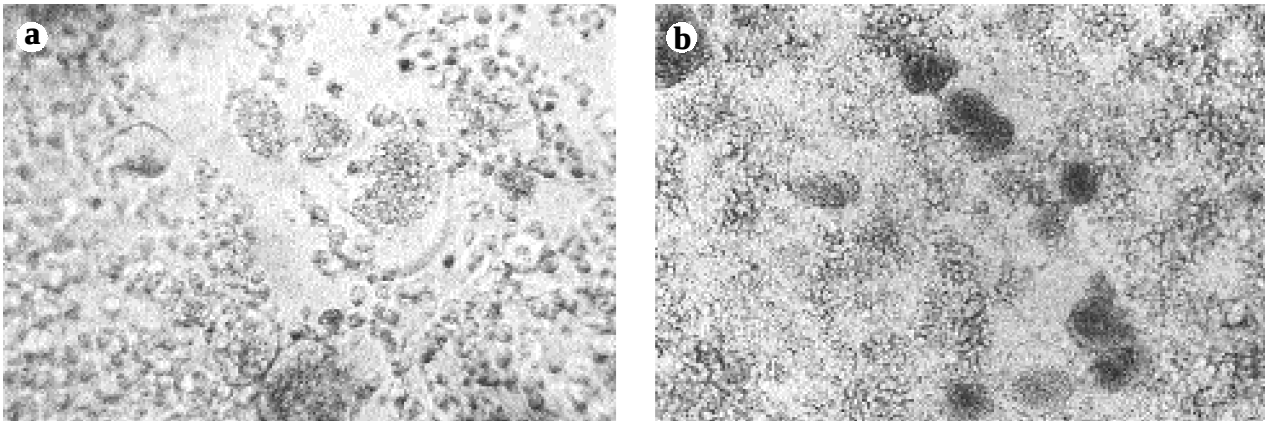

Figure 4. The course of HIV-1/IIIB infection in monolayer CEM-ss cultures under agarose. a. Several syncytia showing balloon degeneration at 72h p.i. (50x). b. Enlarged balloons with condensed cellular content at 96h p.i. (50x).

methylcellulose on day 6 p.i. followed by the sudden collapse with residual dark granules (*Figure 4 b*). Uninfected immobilized cells showed no sign of degeneration at the same time. The course of infection by both HIV-1 strains in CEM and MT-4 cultures followed the same pattern, but the largest syncytia of MT-4 cells were detected on day 7 p.i.

VSV infection of C8166 cells in suspension resulted in enlarged, shiny, smooth cells, which lysed within 48h similarly to those grown in monolayer under a semisolid overlay. VSV infection induced the same morphologic phenomenon in AdCEM cultures, and CEM, MT-4, H9 cells being in either suspen-sion or PLL-bound monolayer with or without overlays.

Electron microscopic studies on the syncytia and HIV-1 produced by C8166 cells

Electron microscopy revealed transiently existing cytoplasmic constituents during the early period of HIV-1 infection of C8166 cells. Samples obtained at 24h p.i. showed two types of cytoplasmic vacuoles. In one of them, which were surrounded with a well definied membrane, few intact virions were localized together with another round bodies or debris of unknown origin (*Figure 5 a*). Virus budding from the membrane of these vacuoles has never been observed. This type of vacuoles containing virions also were seen, if C8166 cell clusters had been dispersed by trypsinization, and the cytopathic effect of HIV-1 with consequent balloon degeneration was strongly diminished and delayed. The other type of vacuoles has never contained any viral or other particles, they were partially surrounded by parts of the Golgi apparatus, and their overall appearance resembled the Birbeck granule of Langerhans cells (*Figure 5 b*). Very early p.i. the fusion of infected cells also showed two different patterns. In one form, enlarging areas of aligned plasma membranes of adjacent cells fused, which always occured in the vicinity of nucleus of the cells (*Figure 5 c*). In the other form, cytoplamic projections fused on a small spot, which places have not been localized very near to nuclei (*Figure 5 d*), and from the tiny fused area no further fusion evolved. Since these protrusions seen microscopically as „hairs“ disappeared with time, the fused protrusions disconnected. Interestingly, no HIV-1 particles were detected in the area of both type of fusions. Usually adjacent to the nucleus (*Fig 5 d-e*), but occassio-nally elsewhere in the cytoplasm (*Figure 5 f*) fine filamentous or lamellar structures were detected that resembled those found in Sézary cells or hairy leukemia cells. These latter structures also disappeared when ballooning dominated cellular structure. During the later phase, particularly from day 3 onwards multiple buds at regular distances were characteristic on the syncytial surface above vacuoles (*Figure 6 a*), but not above nuclei. The same time, the third type of cytoplasmic vacuoles developed, which contained extremely high number of entrapped virions (*Figure 6 b*). Budding from their surrounding membrane was frequently detected, their distribution seemed to be random, and several double buddings were recorded. Viruses budding from the cytoplasmic or vacuolar membranes were similar and showed the characteristics of lentiviruses. But among virions released from the cytoplasmic membrane, several empty particles lacking the dense nucleocapsid core and consisting of the lipid bilayer as well as exhibiting aberrant shape were detected (*Figure 6 e*). In contrast, beside normal particles with the typical conical nucleocapsid released into the cytoplasmic vacuoles, a surprisingly high proportion of „twin“ particles were seen: they contained two dense nucleocapsids in a fused common envelope (*Figure 6 d*).

Discussion

C8166 cells exhibited several new features in response to altered culture conditions and infection. The higher buffer capacity and elevated Ca^{++} level in EMEM as com-

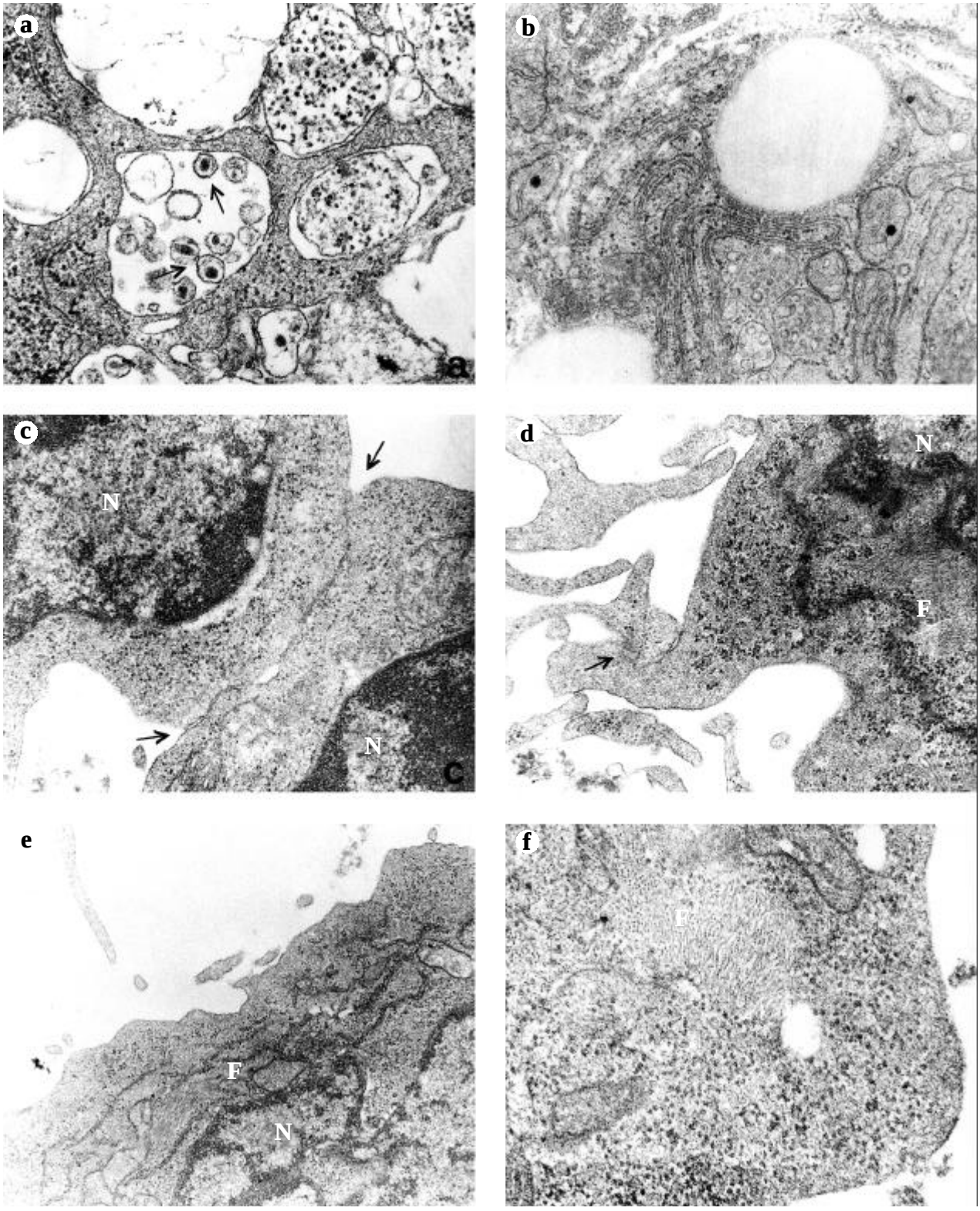

Figure 5. Electron micrograph of HIV-1/RF infected C8166 cells at 24h p.i. a. A coated vesicle containing multiple intact HIV-1 particles (very fine arrows, 60,000x). b. A vesicle resembling Birbeck granule of Langerhans cells (40,000x). c. Fusion of cells membranes (between fine arrows) near nuclei (N) in the absence of vacoules (40,000x). d. Complete fusion of cytoplasmic projections (very fine arrow inside protrusion). d-e. Fibrillar-lamellar structure (F) adjacent to the nucleus (N, 24,000x). f. Fibrillar-lamellar material in the cytoplasm (F, 60,000x).

pared to that of RPMI facilitated plastic adherence of both C8166 and MT-4. This suggests at least a partial activation of their latent monocyte potentials. In the lack of proper stimulation no further cell division took place. Infected single C8166 and MT-4 cells in EMEM did not adhere to culture vessels. It has been described that adherence of HIV-1 infected macrophages is diminished.[5] Other lymphoid cultures, either uninfecter or infected, studied here did not adhere to the plastic. Morphological signs presented here and DNA fragmentation (Ongrádi et al., unpublished) indicates that both infected C8166 and CEM-ss undergo apoptosis followed by syncytium formation upon HIV-1/RF or HIV-1/IIIB infection. High Ca^{++} content is known to enhance both cell fusion[21] by facilitating Ca^{++} influx and apoptosis by stimulating endonuclease activity.[6,22,23] HIV-1 production by C8166 and H9 cultures is higher in EMEM than in RPMI[30,33] suggesting that HIV-1 takes an advantage of altered ionic environment.[6] HTLV-I also induces hypercalcaemia in leukemic patients,[16,18] which might be another factor in its HIV-transactivating effect *in vivo.*

Acting through cellular transcriptional factors, the *tax* polypeptide of HTLV-I enhances replication of HIV-1,[8] regulates cytokine expression,[1] activates lymphocytes to dedifferentiate and replicate,[41] mediates leukemogenesis.[13,16,19,28] The „hairs“ on C8166 cells resemble those found in the blood of patients with hairy leukaemia.[47] In a rare form of this malignancy, HTLV-II is known to transform $CD4^+$ helper T lymphocytes, but in the majority of cases HTLV-I transformed hairy cells express B cell mark-

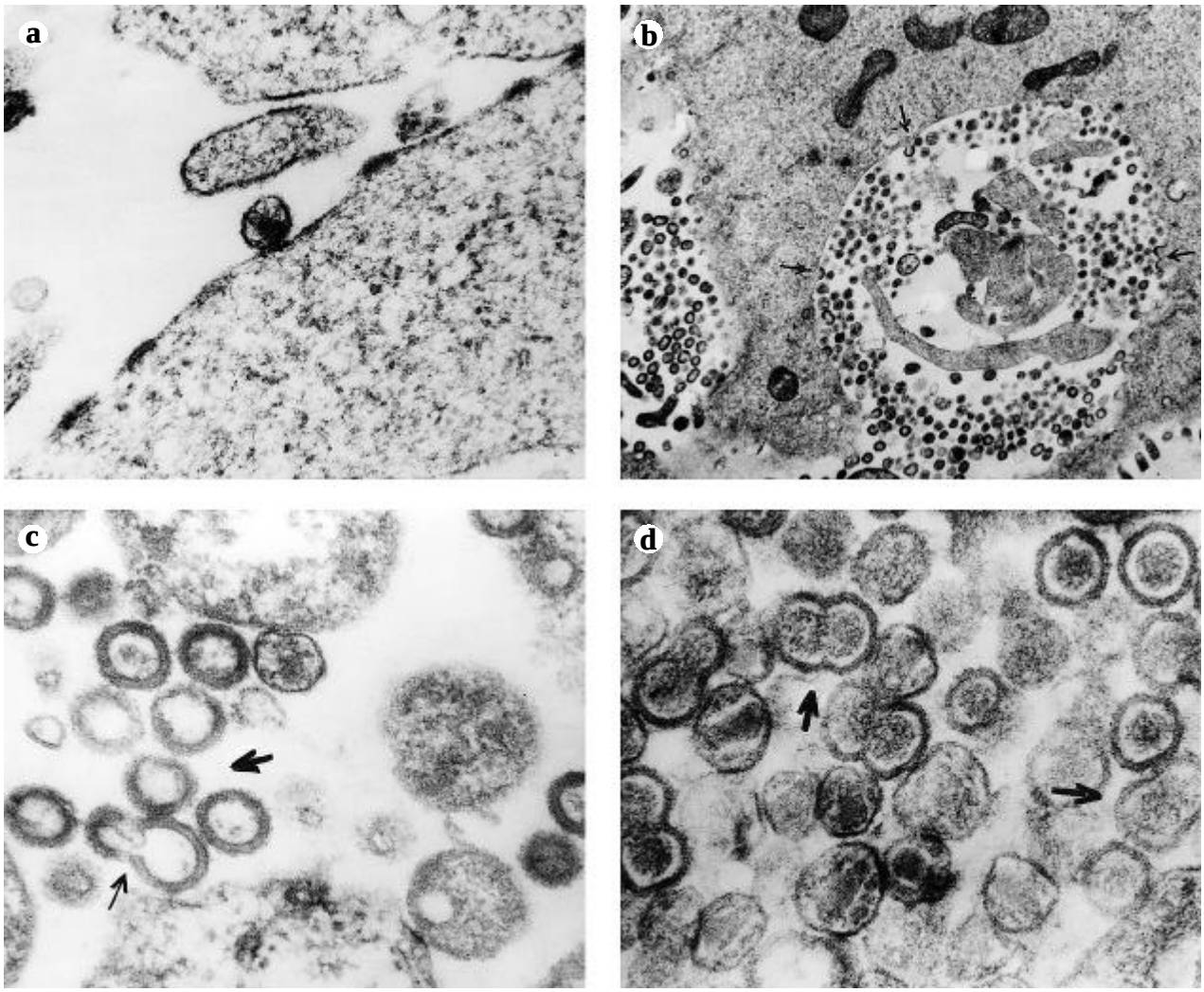

Figure 6. Electron micrograph of HIV-1/RF production by C8166 cells at 96h p.i. a. Surface fragment of a syncytium near a vacuole (V) showing multiple buds and an extracellular HIV particle (70,000x). b. A cytoplasmic vacuole with viruses budding from the membrane (very fine arrows) and released into the vacuole (7000x). c. Empty particles (thick arrow) among them an aberrant particle (fine arrow) released from the cell surface (95,000x). d. Aberrant "twin" virions (thick arrows) released into the cytoplasmic vacuole (95,000x).

ers, avid Fc receptors, and like macrophages, adhere to surfaces, possess a non-specific esterase and phagocytic ability. These cells are pluripotent precursors remaining capable of expressing heterologous cell markers.[16,38] Fc receptors[27] and an esterase[43] of C8166 correspond with these criteria. HIV-1 is known to activate HTLV-I,[1] which event also might occur transiently in the early phase of HIV-1 replication in C8166 cells. The perinuclear lamellar or filamentous structures detected in HIV-1 infected C8166 cells resemble those found in Sézary cells.[47] This disorder is a clonal neoplasm of the $CD4^+$ helper T lymphocytes carrying an integrated HTLV-I. Due to profound epidermotropism it is called as cutaneous T-cell lymphoma. These cells produce several cytokines in the skin, which activate macrophages and enhance the antigen presenting capabilities of Langerhans cells.[8]

Soluble factors acting in an autocrine manner or direct effects upon the early genetic inter-action between HIV-1 and HTLV-I in C8166 cells might elicit formation of Birbeck granule-like structures characteristic for epidermal but not for mature Langerhans cells.[26] These structures appear in the interdigitating dendritic cells derived from $CD34^+$ precursors by GM-CSF and TNF-α activation *in vitro*, if cells are resuspended in liquid but not in semisolid medium[4]. Interdigitating dendritic cells expressing Fc receptors capture HIV-1 and retain them in vesicles encircled by cell surface projections.[3,37,46] This occurs without functional CD4 and coreceptors, is trypsin-resistant and, independently of and simultaneously with productive infection results in a cell-to-cell transfer of HIV-1 to T lymphocytes.[3,25,35,36] Follicular dendritic cells have similar functions.[44] In our C8166 cells, HIV-1 particles were detected in coated vesicles, phenomenon which also occurred in spite of preceding trypsinization and consequently diminished HIV-1 replication. Transmission of HIV-1 is known to be polarized e.g. in epithelial cells, lymph nodes, etc,[23] and might be analogous to highly localized antigen presentation[26] or cytokine release.[34] Also detected in C8166/RF cultures here, virions are rarely observed in fusing membranes of cells, the nuclei form a ring around central Golgi zones,[40] and both surface and intravacuolar buddings of HIV-1 occur on the opposite pole of syncytia. Similarly to certain growth factor receptors and the VSV G envelope protein, several retroviruses, among them both HIV and both HTLV types contain a conserved cytoplasmic domain in their envelope glycoproteins as a polarization signal to direct polarized budding and cell-to-cell spread.[24] Axon-like projections of immobilized C8166/RF cells shown here in the absence of hydrodynamic factors resemble that of HIV-1 infected macrophages[12,23]. Both these and the „hairs“ on the surface of suspended C8166/RF cells might transmit HIV-1 to neighbouring cells. HIV-1 induced axon-like projections of macrophages can transmit virus into physically distant cells of the central nervous system and spleen, whereas APJ, an orphan seven-transmembrane domain receptor serves as an alternative coreceptor for HIV-1 infection.[10] The expression of APJ on C8166 cells might be the consequence of HTLV-I *tax* activity. This virus is known for its neurotropism with strong *tax* expression.[1,17] APJ as a coreceptor on C8166 might contribute to its high HIV-1 sensitivity. Upon VSV infection, lymphoid cells rapidly enlarged with a following necrotic lysis, that also occurs in fibroblasts.[32] VSV did not induce “hairs” or any of the particular alterations mentioned above. Its rapid shut off of host cell macromolecular synthesis and lack of interactions with cellular and proviral DNA[6] might prevent the effects of its polarization signal.

C8166 cells can be infected by lymphotropic HIV-1 strains only.[5,6,8,15,20,27] Viruses produced are released by both cytoplasmic and intravacuolar budding,[15,19,20] which latter is characteristic for macrophages and dendritic cells, serving the silent distribution of virus.[12,23] HIV-1/IIIB is known to replicate in chimpanzee macrophages,[11] both HIV-1/IIIB and RF productively infect U937 promonocytic cells.[5] This suggests that lymphotropic HIV-1 strains can utilize the replicative pathway of monocytotropic strains. Virions entrapped in vacuoles escape when the cells disintegrate, but the acidic medium destroys their infectivity.[31] The high proportion of empty particles released early from the cytoplasm and that of „twin“ or multiple cored particles released relatively late might have different biological functions. Gp120 polypeptide in the viral envelope can induce syncytium formation[43] and apoptosis[26] (Ongrádi et al., unpublished) in physically distant cells, while the viral genomic RNA can be transmitted via cell-to-cell into special target T cells to multiply.[3,25,35,37,40] Excess genomic RNA might be packed into multicored particles, or HTLV-I might take part in their production. The biological significance of the 4, 6, or more complete or defective genomes in a common envelope is unknown.

Both HTLV-I and II have entered indigenous populations world wide, are present predominantly amongst intravenous drug users and are usually coinfections with HIV-1. Unusual leukemias and higher susceptibility for neurological disorders have already been reported and their number is expected to increase in near future.[1,13,17,45] Pluripotent C8166 cells might serve as an *in vitro* model to study interaction among HTLV, HIV and host cells.

Acknowledgement

This study was supported by grant 201/1 from the Hungarian AIDS Committee, grants T-020489 and T-29299 from the Hungarian Scientific Research Fund, a grant from the British Technology Group, grants from the AIDS Program of the Italian Institute for Public Health. AdCEM, C8166, H9 and MT-4 cells as well as HIV-1/RF were obtained from R. A. Weiss (Institute for Cancer Research, London, UK), CEM-ss cells and HIV-1/IIIB were gifts

from R. C. Gallo (National Institute of Health, Bethesda, MD, US). Technical help of Alex Orr and Lucia Barontini, as well as photographic artwork by Alberto Bertini are highly appreciated.

References

1. *Beilke MA, Japa S, Vinson DG*: HTLV-I and HTLV-II virus expression increase with HIV-1 coinfection. J. Acquir. Immune Defic. Syndr. Hum. Retrovir. 17:391-397, 1998.
2. *Bjornson CRR, Rietze RL, Reynolds BA, et al*: Turning brain into blood: A hematopoietic fate adopted by adult neural stem cells in vivo. Science 283:534-537, 1999.
3. *Blauvelt A, Asada H, Saville MW, et al*: Productive infection of denritic cells by HIV-1 and their ability to capture virus are mediated through separate pathways. J. Clin. Investig. 100:2043-2053, 1997.
4. *Charbonnier AS, Verrier B, Jacquet C, et al*: *In vitro* HIV1 infection of $CD34^+$ progenitor-derived dendritic/Langerhans cells at different stages of their differentiation in the presence of GM-CSF/TNF. Res. Virol. 147:89-95, 1996.
5. *Clapham PR, Weiss RA, Dalgleish AG et al:* Human immunodeficiency virus infection of monocytic and T-lymphocytic cells: receptor modulation and differentiation induced by phorbol ester. Virology 158:44-51, 1987.
6. *Cloyd MW, Lynn WS:* Perturbation of host cell membrane is a primary mechanism of HIV cytopathology. Virology 181:500-511, 1991.
7. *De Vita VT, Hallman S and Rosenberg SA:* Cancer. 5th ed., Lippincott-Raven Publ., Philadelphia, 1997.
8. *Dianzani F, Antonelli G, Capobianchi MR, De Marco F*: Replication of human immunodeficiency virus: yield of infectious virus under single growth cycle conditions. Arch. Virol. 103:127-131, 1988.
9. *Dolei A, Biolchini A, Serra C, et al*: Increased replication of T-cell-tropic HIV strains and CXC-chemokine receptor-4 induction in T cells treated with macrophage inflammatory protein (MIP)-1, MIP-1 and RANTES -chemokines. AIDS 12:183-190, 1998.
10. *Edinger AL, Hoffman TL, Sharron M, et al*: An orphan seven-transmembrane domain receptor expressed widely in the brain functions as a coreceptor for human immunodeficiency virus type 1 and simian immunodeficiency virus. J. Virol. 72:7934-7940, 1998.
11. *Eibl MM, Küpcü Z, Mannhalter JW, et al*: Dual tropism of HIV-1 IIIB for chimpanzee lymphocytes and monocytes. AIDS Res. Human Retrovir. 8:69-75, 1992.
12. *Gendelman HE, Orenstein JM, Baca LM, et al*: The macrophage in the persistence and pathogenesis of HIV infection. AIDS 3:475-495, 1989.
13. *Hamaia S, Cassé H, Gazzolo L, et al:* The human T-cell leukemia virus type 1 rex regulatory protein exhibits an impaired functionality in human lymphoblastoid Jurkat T cells. J. Virol. 71:8514-8521, 1997.
14. *Haraguchi Y, Takeuchi Y, Hoshino H:* Inhibition of plating of human T cell leukemia virus type I and syncytium-inducing types of human immunodeficiency virus type 1 by polycations. AIDS Res. Hum Retrovir 13:1517-1523, 1997.
15. *Harris JR, Kitchen AD, Harrison JF, et al*: Viral release from HIV-1-induced syncytia of $CD4^+$ C8166 cells. J Med Virol 28:81-89, 1989.
16. *Henderson ES, Lister TA, Greaves MF*: Leukemia, 6th ed., WB. Saunders Co., Philadelphia, 1996.
17. *Hildreth JEK, Subramanium A, Hampton RA*: Human T-cell lymphotropic virus type 1 (HTLV-1)-induced syncytium formation mediated by vascular cell adhesion molecule-1: Evidence for involvement of cell adhesion molecules in HTLV-1 biology. J Virol 71:1173-1180, 1997.
18. *Hoffman R, Benz Ejjr, Shattil SJ, et al*: Haematology, 2nd ed., Churchill-Livingstone, New York, 1995.
19. *Kiernan R, Marshall J, Bowers R, et al:* Kinetics of HIV-1 replication and intracellular accumulation of particles in HTLV-I transformed cells. AIDS Res. Human Retrovir. 6:743-752, 1990.
20. *Kinchington D, Barker W, Galpin S, et al:* Temperature enhancement of syncytium formation by HIV and Sendai virus. J Med Virol 36:44-48, 1992.
21. *Larsen CE, Alford DR, Young LJ, et al:* Fusion of simian immunodeficiency virus with liposomes and erythrocyte ghost membranes: effects of lipid composition, pH and calcium. J Gen Virol 71:1947-1955, 1990.
22. *Laurent-Crawford AG, Krust B, Muller S, et al.*: The cytopathic effect of HIV is associated with apoptosis. Virology 185:829-839, 1991.
23. *Levy JA:* HIV and the pathogenesis of AIDS. ASM Press, Washington, 1998.
24. *Lodge R, Delamarre L, Lalonde J-P, et al*: Two distinct oncornaviruses harbor an intracytoplasmic tyrosine-based basolateral targeting signal in their viral envelope glycoprotein. J Virol 71:5696-5702, 1997.
25. *Ludewig B, Gelderblom HR, Becker Y, et al*: Transmission of HIV-1 from productively infected mature Langerhans cells to primary CD4+ T lym-phocytes results in altered T-cell responses with enhanced production of IFN-γ and IL-10. Virology 215:51-60, 1996.
26. *Ludewig B, Holzmeister J, Gentile M, et al*: Replication pattern of human immunodeficiency virus type 1 in mature Langerhans cells. J Gen Virol 76:1317-1325, 1995
27. *McLain L, Dimmock NJ:* A human $CD4^+$ T-cell line expresses functional CD64 (FcγRI), CD32 (FcγRII), and CD16 (FcγRIII) receptors but these do not enhance their infectivity of HIV-1-IgG complexes. Immunology 90:109-114, 1997.
28. *Nagata K, Ide Y, Takagi T, et al.*: Complex formation of human T-cell leukemia virus type I $p40^{tax}$ transactivator with cellular polypeptides. J Virol 66: 1040-1049, 1992.
29. *Nowotny A:* Basic excercises in immunochemistry. Springer-Verlag, Berlin-Heidelberg-New York, 1979.
30. *Ongrádi J, Ceccherini-Nelli L, Szilágyi JF, et al*: The influence of cell culture and storage conditions on HIV-1 infectivity and fusogenic activity. Acta Microbiol Hung 39:207-221, 1993.
31. *Ongrádi J, Ceccherini-Nelli L, Pistello M, et al*: Acid sensitivity of cell-free and cell-associated HIV-1: Clinical implications. AIDS Res. Human Retrovir 6:1433-1436, 1990.
32. *Ongrádi J, Cunningham C, Szilágyi JF*: The role of polypeptides L and NS in the transcription process of vesicular stomatitis virus New Jersey using the temprature sensitive mutant *ts*E1. J Gen Virol 66:1011-1023, 1985.
33. *Ongrádi J, Szilágyi JF, Laird HM, et al*: Studies on the course of HIV-1 infection in tissue cultures. Hung Med J 134:803-809, 1993.
34. *Poo W-J, Conrad L, Jeneway CA:* Receptor-directed focusing of lymphokine release by helper T cells. Nature 332:378-380, 1988.
35. *Pope M, Betjes MGH, Romani N, et al*: Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. Cell 78:389-398, 1994.
36. *Ramazzotti E, Marconi A, Re MC, et al*: *In vitro* infection of human epidermal Langerhans' cells with HIV-1. Immunology 85:94-98, 1995.
37. *Randolph GJ, Beaulieu S, Lebecque S, et al*: Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science 282:480-483, 1998.

38.²*Rubin E, Farber JL*: Pathology, J.B. Lippincott Co., Philadelphia, 1988.
39.²*Salahuddin SZ, Markham PD, Wong-Staal F, et al*: Restricted expression of human T-cell leukemia-lymphoma virus (HTLV) in transformed human umbilical cord blood leukocytes. Virology 129: 51-64, 1983.
40.²*Sato H, Orenstein J, Dimitrov D, et al:* Cell-to-cell spread of HIV-1 occurs within minutes and may not involve the participation of virus particles. Virology 186:712-724, 1992.
41.²*Sinkovics JG:* Medical Oncology, Vol. I, Marcel Dekker Inc., New York-Basel, 1986, p 126-131.
42.²*Sodroski JG, Rosen CA, Haseltine WA*: *Trans*-acting transcriptional activation of the long terminal repeat of human T lymphotropic viruses in infected cells. Science 225:381-385, 1984.
43.²*Sodroski J, Goh WC, Rosen C, et al*: Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. Nature 322:470-474, 1986.
44.²*Tacchetti C, Favre A, Moresco L, Meszaros P, et al*: HIV is trapped and masked in the cytoplasm of lymph node follicular dendritic cells. Am J Pathol 150:533-542, 1997.
45.²The HTLV European Network: Seroepidemiology of the human T-cell leukaemia/ lymphoma viruses in Europe. J Acquir Immune Defic Syndr Hum Retrovir 13:68-77, 1996.
46.²*Weissman D, Li Y, Ananworanich J, Zhou L-J, et al*.: Three populations of cells with dendritic morphology exist in peripheral blood, only one of which is infectable with human immunodeficiency virus type 1. Proc Natl Acad Sci USA 92:826-830, 1995.
47.²*Zucker-Franklin D, Greaves MF, Grosci CE, Marmont AM:* Le cellule del sangue. Edi-Ermes, Milano, 1988.